

***Gyalecta caudiospora* sp. nov. from China**

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ABSTRACT—*Gyalecta caudiospora* is described from China as a new species. It is characterized by its greenish thallus, lecideine apothecia with pale yellow disc, 8-spored ascus, 3-septate ascospores with long attenuated appendage at the lower end, and the absence of lichen substances. The specimens were deposited in LCU. A key to the *Gyalecta* species recorded in China is presented.

KEY WORDS—*Gyalectaceae*, lichenized fungi, *Ostropales*, taxonomy

Introduction

Gyalecta was introduced by Acharius in 1808 and has a cosmopolitan distribution on a wide range of substrates (Lücking 1999, Alvarez Andrés & López de Silanes 2002, Gagarina 2011, Aptroot & Moon 2014). The genus is characterized by its crustose thallus, lecideine apothecia, simple paraphyses, 8-spored asci, and transversely septate or submuriform to muriform ascospores.

Gyalecta includes more than 50 species worldwide (Kirk & al. 2008, Baloch & al. 2013, Jaklitsch & al. 2016). Two species: *G. alutacea* Zahlbr. reported from Yunnan (Zahlbruckner 1930, Wei 1991) and *G. foveolaris* (Ach.) Schaer. from Xizang (Obermayer 2004) are known in China. During our study of *Gyalecta*, we collected specimens that differed from previously described species and propose them here as a new species, *G. caudiospora*.

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TABLE 1. Absolute distances between alignment sequences of nrLSU region from *Gyalecta caudiospora* and related species.
(Gaps ignored in pairwise comparisons.)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. <i>G. caudiospora</i> MH345767														
2. <i>G. herculana</i> FJ941886	44													
3. <i>G. jenensis</i> 1 KR017187	52	45												
4. <i>G. jenensis</i> 2 AY340544	51	49	19											
5. <i>G. jenensis</i> 3 AF465450	49	47	19	2										
6. <i>G. jenensis</i> 4 AF279391	51	47	16	5	5									
7. <i>G. friesii</i> 1 KJ766566	61	66	77	71	71	71								
8. <i>G. friesii</i> 2 HQ659179	61	66	77	71	71	71	0							
9. <i>G. hypoleuca</i> AF465453	51	43	59	59	59	57	68	68						
10. <i>G. truncigena</i> AF465451	60	60	75	76	76	76	48	48	59					
11. <i>G. thelotremella</i> AF465455	46	28	53	59	57	57	65	65	32	58				
12. <i>G. herrei</i> AF465449	49	19	51	56	54	54	70	70	40	59	28			
13. <i>G. ulmi</i> AF465463	59	56	71	68	68	68	26	26	58	50	54	59		
14. <i>G. leucaspis</i> AF465462	72	71	83	76	76	76	50	50	71	64	76	74	44	
15. <i>R. rhexoblephara</i> KJ766656	68	65	79	76	76	75	51	51	71	64	66	68	43	61

* Intraspecific distances are indicated with bold font.

Materials & methods

SPECIMENS. All materials were collected from Guizhou Province, China, and are deposited in Lichen Herbarium of the College of Life Sciences, Liaocheng University, Liaocheng, China (LCU). Morphological characters were examined and photographed under TECH XTS-30D and Olympus SZX16 dissecting microscopes. The anatomical characters were examined and photographed under an Olympus BX53 compound microscope. Cortex and medulla were tested using K (a 10% aqueous solution of potassium hydroxide), C (a saturated solution of aqueous sodium hypochlorite), and P (a saturated solution of *p*-phenylenediamine in 95% ethyl alcohol). The lichen substances were detected using standardized thin layer chromatography (TLC) (Orange & al. 2010; Jia & Wei 2016).

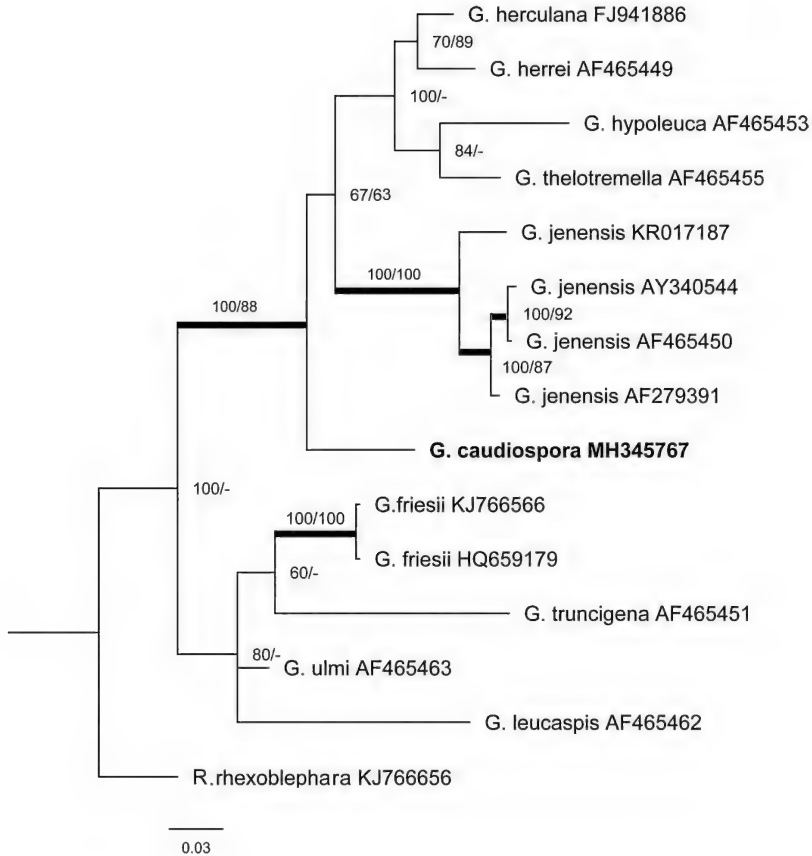


FIG. 1. Phylogenetic relationships inferred from nrLSU sequences of *Gyalecta caudiospora* and closely related *Gyalecta* species (with *Rhexophiale rhexoblephara* as outgroup). The evolutionary tree was inferred by using the Bayesian method based on the GTR+I+J model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Posterior probabilities/ML-bootstrap values above 50% are written next to nodes. Branches highly supported by both methods are indicated with a bold line.

DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING. Genomic DNA was extracted from ascomata of the type specimen using the Hi-DNAsecure Plant Kit according to the manufacturer's protocol. The partial region of nuclear large unit rDNA (nuLSU) was amplified using the AL2R and LR6 primers (Mangold & al. 2008, Vilgalys & Hester 1990) in 25 μ L reaction system containing 1 μ L each primer solution (10 μ M), 0.5 μ L genomic DNA, 10 μ L ddH₂O, and 12.5 μ L 2 $\times$ Taq PCR MasterMix[®]. Thermocycling conditions comprised initial denaturation at 94 $^{\circ}$ C (5 min); 35 denaturation cycles at

95°C (30 s), annealing at 58°C (30 s), extension at 72°C (1 min), and a final extension at 72°C for 10 min. The target product of PCR was affirmed by electrophoresis on 1% agarose gels and sequenced by Biosune Inc. (Shanghai).

The newly generated sequence was submitted to GenBank and aligned with nuLSU sequences representing similar taxa (FIG. 1). These taxa were selected based on the results of nuLSU sequence Blast searches, morphological characters, and related literature (Baloch & al. 2010).

PHYLOGENETIC ANALYSIS AND SEQUENCE COMPARISONS. The nuLSU sequences from our specimen and 14 selected references were aligned by Muscle using MEGA5 (Tamura & al. 2011). The final matrix can be obtained from the corresponding author.

A phylogenetic tree was generated using Bayesian inference (PP) method based on GTR+I+G model with 6 rates = Invgamma and Maximum Likelihood (ML) method based on the Tamura-Nei model (Tamura & Nei 1993) in MEGA5. The confidence level in the resulting topological bipartitions was estimated with 1000 bootstrap replicates. All alignment gaps and missing data were excluded during phylogenetic analyses via pairwise sequence comparisons. The analyses encompassed 15 nucleotide sequences. Absolute distances were estimated using MEGA5, based on p-distance model with all gaps removed from each sequence pair.

Sequencing & phylogenetic results

The nrLSU partial region from the Guizhou sample was successfully sequenced; the sequence submitted to GenBank contained 953 base pairs. Any sites that were difficult to align were removed from the matrix, reserving 695 total positions for phylogenetic analyses in the final dataset.

Blast searches of nrLSU sequences indicated *G. caudiospora* has close affinities with *G. herrei* (93% identity), *G. herculana* (93% identity), and *G. ulmi* (91% identity). The phylogenetic analyses strongly supported the monophyly of *G. jenesi* (PP = 100%; ML = 100%) and *G. friesii* (PP = 100%; ML = 100%). *Gyalecta caudiospora* did not group with any other species in the tree.

Absolute distances for the aligned sequences of the nrLSU partial region also support the separation of a new species. In our sequence matrix, distances between infraspecific samples are ≤ 19 , while distances between species are ≥ 19 (TABLE 1).

Taxonomy

***Gyalecta caudiospora* Z.F. Jia, sp. nov.**

FIG. 2

FUNGAL NAME FN 570572

Differs from *Gyalecta ancistrospora* by its smaller apothecia, pale yellow and concave disc, and narrower ascospores.

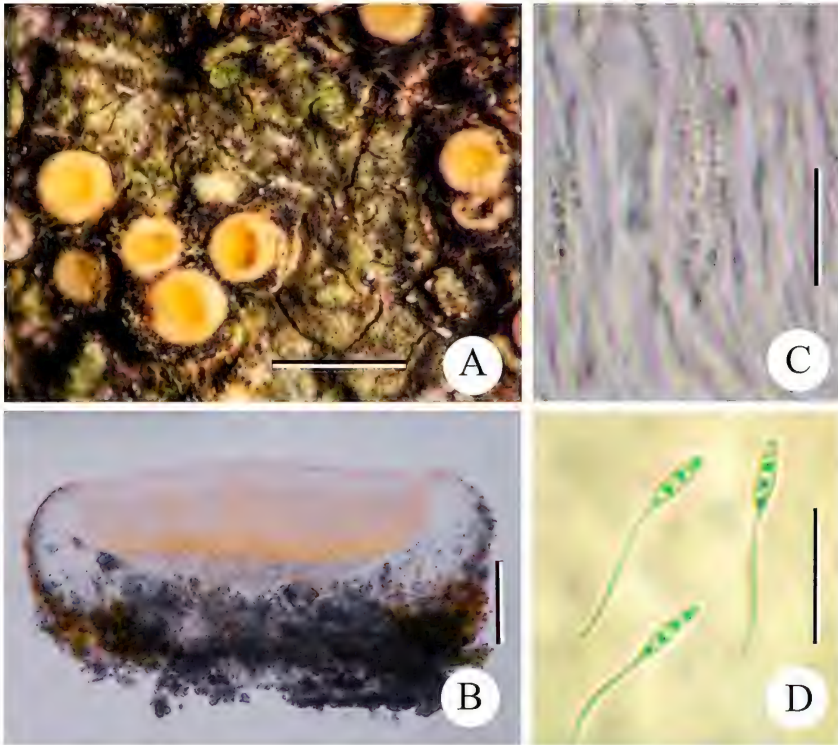


FIG. 2. *Gyalecta caudiospora* (holotype, LCU: X.H. Wu GZ17001). A. Thallus with apothecia; B. Cross section of apothecium; C. Ascus with ascospores; D. Ascospores. Scale bars: A = 1 mm; B = 50 μ m; C, D = 20 μ m.

TYPE: China. Guizhou: Xingyi City, Zhengtun Town, Minzu Village, Geyser, 25°06'N 105°06'E, alt. 1240 m, on rock, 1/X/2017, X.H. Wu GZ17001 (Holotype, LCU; GenBank MH345767).

ETYMOLOGY: derived from the Latin *caudatus* + *spora* referring to the long thin appendage on the ascospore.

THALLUS crustose, saxicolous, army greenish, dull. Algae trebouxoid, c. 10 $\times$ 5 μ m. APOTHECIA lecideine, sessile, 0.15–0.35 mm diam.; DISC concave, pale yellow, epruinose, margin pale, higher than the disc; EXCIPLE hyaline; EPITHECIUM brownish, 8–12 μ m tall; HYMENIUM 65–80 μ m tall, hyaline, without oil droplets, I+ pale blue, paraphyses simple, 1.5–2.5 μ m diam.; HYPOTHECIUM hyaline. ASCI cylindrical to clavate, 40–50 $\times$ 7.5–9 μ m, 8-spored. ASCOSPORES 25–40 $\times$ 2–2.5 μ m, 3-septate, fusiform, hyaline, with a long, attenuated appendage at the lower end, c. 1.5–2 $\times$ the length of the spore body. PYCNIDIA not observed.

CHEMISTRY: Thallus UV–, C–, K–, KC–, P–. No lichen substances detected with TLC.

ECOLOGY & DISTRIBUTION: On rocks in forest. Known only from the type locality.

ADDITIONAL SPECIMEN EXAMINED: China. Guizhou: Xingyi City, Zhengtun Town, Minzu Village, Geyser, alt. 1240 m, on rock, 1/X/2017, X.H. Wu GZ17002 (LCU).

REMARKS: *Gyalecta caudiospora* morphologically resembles *G. ancistrospora* Aptroot & K.H. Moon, which differs by its pale greenish grey thallus, its larger apothecia (0.3–0.6 mm) with orange flat disc, and its wider ascospores (3.5–4 µm; Aptroot & Moon 2014).

Both *G. alutacea* and *G. foveolaris* reported in China have smaller ascospores without appendages (Zahlbruckner 1930, Obermayer 2004).

Previous reports that used DNA sequence data to support species delimitation in lichens required both monophyly in phylogeny and diagnostic morphology (Ababaikeli & al. 2016). Our molecular and morphological data of *G. caudiospora* match these requirements for new species recognition.

Key to the species of *Gyalecta* known from China

1. Ascospores small, 5–6 × 1 µm *G. alutacea*
1. Ascospores larger, >10 µm long 2
2. Ascospores 12–16 × 5–6 µm; without appendages *G. foveolaris*
2. Ascospores 25–40 × 2–2.5 µm; with a lower appendage *G. caudiospora*

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